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(71)(72) Applicants and Inventors: NILSSON, Kurt [SE/SE]; Andjaktsv. 6, S-226 53 Lund (SE). MANDENIUS, Carl-Fredrik [SE/SE]; Strömkarlsv. 36, S-141 42 Huddinge (SE).

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(54) Title: IMMOBILIZED CARBOHYDRATE BIOSENSOR

(57) Abstract

The present application refers to a biosensor in which an immobilized carbohydrate or a derivative thereof is used to generate a detectable signal when a protein, a virus or a cell is bound to the carbohydrate surface. The sensor is an optical sensor, a piezoelectric sensor, an electrochemical electrode or a thermistor. A method of binding carbohydrates to a gold surface is also described.

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Immobilized carbohydrate biosensor

The present invention relates to a biosensor in which a carbohydrate or a derivative thereof is used to generate a detectable signal via the specific binding of a protein, a virus or a cell.

Background.

Biosensors are characterised by a physical or chemical signal transducer, which response is activated by a specific interaction between a biochemical structure (which directly or indirectly has been bound to the transducer) and one or several analytes.

Biosensors are used to detect the analyte/analytes and in certain cases also for quantification of the analyte/analytes.

The advantages of the biosensor are that a physical or chemical transducer has been made specific so that a general physical or chemical parameter (e.g. temperature, pH, optical density) can be used for the detection of one specific substance in a complex mixture of non-specific substances.

The limitations of the biosensor are the specificity of the biochemical structure bound to the transducer, the range of specificity and stability, and, that the transducer signal has to be made independent of the background changes in the parameter that the transducer is measuring. In Methods of Enzymology, volume 137, several articles are describing different aspects of biosensors.

Definitions.

Biosensor - physical or chemical signal transducer, e.g. photometer, chemical electrode, temperature or pressure signal transducer, which directly or indirectly has been connected with a biochemical structure. In previous biosensors one has preferentially used an enzyme, a specific protein or antibody as the biochemical structure and in this way the biosensors have been given the property of being able to detect substances which specifically bind to the biochemical structure in a qualitative or quantitative way.

Reflection measurement - measurement of the intensity of light reflected from a surface where the properties of the surface influences the reflection, e.g. biomolecules which change the refraction index of the surface.

Polarisation measurement - measurement of the polarisation of polarised light, usually as the angle of polarisation, which is depending on the binding of biomolecules, virus or cells.

Surface plasmon spectroscopy - optical physical measurement technique which utilise the surface plasmon condition of thin metal surfaces, which can be used to measure small changes of refraction index with high sensitivity, e.g. as caused by the presence of biomolecules on the surface.

Ellipsometry - optical physical measurement technique which can be used to measure small changes of refraction index at surfaces with high sensitivity, by measuring changes in elliptisity of polarised light, e.g. as caused by the presence of biomolecules on the surface.

Piezoelectric crystal - crystal which frequency can be influenced by changes of mass or pressure which can be measured electrically, for example the change of mass caused by the presence of biomolecule(s), virus or cell(s) bound to the crystal surface.

Electrochemical electrode - measuring device which generates an electrical signal caused by an electrochemical reaction at the electrode which is related to a chemical parameter, e.g. pH, pO_2, pCO_2, the values of which can vary because of the presence of analyte(s) in a sample specific for a compound bound to the measuring device.

Thermistor - electrical resistance device which changes resistance with the temperature; biochemical reactions are characterised by e.g. specific values of heat consumption/formation, which can be registered via the thermistor.

A large amount of the carbohydrate sequences present in glycoproteins or in glycolipids, and usually also smaller fragments of these sequences, have shown biospecific binding to proteins, virus or cells.

The present invention describes a biosensor where this specificity is used for determination of such a component in a sample. The invention is characterised by that the carbohydrate or a derivative thereof is bound to a surface in the biosensor.

As carbohydrate, one can use fragments (oligosaccharides) of the carbohydrate sequences found in glycoproteins or in glycolipids and one can also use smaller fragments of these sequences, i.e. disaccharide, trisaccharide, tetrasaccharide or a pentasaccharide, because this size usually is sufficient for the oligosaccharide to bind a protein, virus or a cell in a biospecific manner. A review or such carbohydrate sequences can be found in e.g. Chemistry and Physics of Lipids, vol. 42, p. 153 -172, 1986, and in Ann. Rev. Biochem., vol. 58, p. 309-350.

The oligosaccharide is usually modified in the reducing end with an aglycon, which is composed of a glycosidically bound organic group which is suitable for binding to the surface in the biosensor. Examples of aglycons are $OEtSEtCONHNH_2$, -$OEtSPhNH_2$, etc. The binding to the surface in the biosensor can be done directly or via a proteins, e.g. bovine serum albumine or via a chemical structure which has been adsorbed or which has been covalently bound to the surface. Such a chemical structure can contain reactive organic groups such as carboxyl-, sulfonate, cyanate, epoxy-, aldehyde groups or other groups suitable for chemical conjugation with for example an amine or thiol group in the aglycon.

More specific examples of analytes which can be analysed with biosensor according to the present invention are lectins, antibodies against carbohydrates, pathogenic virus or bacteria, such as urinary tract bacteria (e.g. P-fimbriated E. coli) or pathogens of the respiratory tract, and bacteria which cause infections/diarrhea in in gastrointestinal tract.

Non-limiting examples of carbohydrate structures of interest and which can be used in the form of a carbohydrate derivative in a biosensor according to the invention, are monosaccharides, disaccharides, trisaccharides and higher oligosaccharides which show biological activity or which has the ability to specfically bind one or more biomolecules or a group of biomolecules. Examples of biomolecules are other saccharides, peptides and proteins. Examples of such carbohydrate sequences are the blood group determinants (for example A, B, H, Lewis-a, Lewis-b, Lewis-x, Lewis-y), cancer-associated carbohydrate sequences,

In some situations, e.g. to increase the biosensor signal in the measurement of low concentrations of cells, it can be advantageous in the measurement of the analyte with the biosensor to add, after the binding of the analyte to the carbohydrate surface, microparticles modified with carbohydrate specific for the bound cell.

The surface of the biosensor can be, for example a gold surface or a modified gold surface, a plastic surface which has been modified with a gold surface, silver surface or another metallic surface, or modifications thereof with polymers to which chemical coupling of carbohydrate can be carried out.

Below are given non-limiting examples of carbohydrate surfaces which can be used in biosensors according to the invention for binding and analysis/determination of pathogenic bacteria of the urinary tract.

EXAMPLE

One example was performed as follows: Silica surface coated with a gold layer was modified with mercaptopropionic acid by dipping the surface in a 5 mM solution of the acid. The carboxyl groups were modified with carbodiimide (EDC) for 2 hours, whereafter digalactoside with aglycon (Galα1-4Galβ-OEtSEtCONHNH$_2$), was coupled to the EDC-activated surface for 12 hours at pH 8.5 and the surface was then rinsed with buffer.

The thus obtained gold surface modified with digalactoside was dipped for 60 minutes (this time can be varied) in a sample with bacteria of the urinary tract (P-fimbriated E. coli) containing Galα1-4Gal-specific receptor protein, followed by rinsing of the surface with distilled water for 2 minutes. Another gold surface modified in the same way with Galα1-4Gal, was dipped in a sample containing another non-infectious E.coli strain which lack the Galα1-4Gal-specific receptor protein. The extent of binding of the different bacteria to the surfaces was compared with electrom microscopy. The bacteria with The Galα1-4Gal-receptor bound to the surface to a ca 10-15 times higher extent than the other bacteria. The binding of P-fimbriated E. coli to a gold surface modified with mercapto-propionic acid alone, was ca 20 times lower than to the

Galα1-4Gal-modified surface.

Alternative non-limiting examples are given below in which a neoglycoprotein was bound covalently or adsorbed directly on a surface for use in biosensor according to the invention.

In procedure B, Galα1-4GalβOCH$_2$CH$_2$SCH$_2$CH$_2$C(O)-NHNH-BSA was coupled to the same type of EDC-activated gold plate as in the procedure above. The Galabiose-BSA derivative (0.1 mg/ml) was dissolved in 0.1 M boronate, pH 8.5 and EDC-activated plates were immersed in this solution for 1 hour. Subsequently the plates were immersed in a BSA solution (3 mg/ml) in phosphate buffer for 1 minute and rinsed with buffer and distilled water and stored as above.

In procedure C, Gold plates (not pretreated with mercaptopropionic acid) were immersed in a solution of Galα1-4Galβ-BSA (0.1 mg/ml) in 0.1 M sodium phosphate, pH 6.0, for 1 hour and subsequently immersed in the above BSA solution (3 mg/ml) for 1 minute, rinsed with buffer and distilled water and stored as above.

These latter biosensor surfaces showed similar characteristics and low back-ground binding of bacteria as surface in the first example above.

CLAIMS

1. Biosensor, characterised in that at least one carbohydrate derivative with ability to bind a protein, virus or a cell in a sample is bound to a surface in the biosensor.

2. Biosensor according to claim 1 above, characterised in that the carbohydrate derivative is chemically bound or is bound via adsorption to a surface which constitutes one part of the biosensors signal transducer part.

3. Biosensor according to claim 1, where the carbohydrate part of the carbohydrate derivative contains at least one component consisting of hexosamine-, fucose-, galactose- glucose-, mannose-, xylose-, N-acetylneuraminic acid residue or an analog thereof.

4. Biosensor according to claim 1, where the carbohydrate part of the carbohydrate derivative contains at least one component consisting of hexosamine-, fucose-, galactose- glucose-, mannose-, xylose-, N-acetylneuraminic acid residue or an analog thereof, which has been derivatised in at least one of their hydroxyl groups or amino groups with an organic or inorganic group.

5. Biosensor according to one or more of the claims above, in which the carbohydrate derivative contains at least one O-, N-, S-, or C-glycosidically bound aglycon.

6. Biosensor according to one or more of the claims above, in which the aglycon part of the carbohydrate derivative contains at least one alifatic or aromatic compound.

7. Biosensor according to one or more of the claims above, in which the aglycon part of the carbohydrate derivative contains an amino acid-, peptide- or protein component.

8. Biosensor according to one or more of the claims above, in which the carbohydrate derivative consist of a glycoprotein or a neoglycoprotein which is bound covalently or via adsorption to a surface which consist of the signal

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 94/00343

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: G01N 33/543, C12Q 1/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, WPIL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE, C2, 3617763 (OLYMPUS OPTICAL CO., LTD.), 17 August 1989 (17.08.89), see fig 13 and page 25	1-4,10
Y		1-16
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X	EP, A2, 0215669 (SEIKO INSTRUMENTS & ELECTRONICS LTD.), 25 March 1987 (25.03.87), see p. 6 line 19 and claim 6	1-4,10
Y		1-15
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
18 July 1994	29 -07- 1994
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A1, 9001166 (ARES-SERONO RESEARCH & DEVELOPMENT LIMITED PARTNERSHIP), 8 February 1990 (08.02.90), see oages 14 and 4, line 19	1-4,10
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X	US, A, 5082929 (THAT T. NGO ET AL), 21 January 1992 (21.01.92), column 1 - column 2	1-4,8
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INTERNATIONAL SEARCH REPORT

Information on patent family members

02/07/94

International application No.
PCT/SE 94/00343

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		JP-A- 62288546	15/12/87
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